

Data trend shifts induced by method of concentration for trace metals in seawater: Automated online preconcentration vs. borohydride reductive coprecipitation of nearshore seawater samples for analysis of Ni, Cu, Zn, Cd, and Pb via ICP-MS

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Abstract

This research compares performance, reproducibility, and detection limits of ambient seawater analysis for trace metals using both borohydride reductive coprecipitation and an automated chelation column (seaFAST™ 2) preconcentration for matrix interferent elimination on total and dissolved grab samples in nearshore to marine waters, over a broad concentration range, prior to inductively coupled plasma mass spectrometry (ICP-MS) injection. A move to an online preconcentration method both minimizes sample preparation, and eliminates correction errors when accounting for trace impurities in precipitated samples, induced via reagents. The reproducibility of the online preconcentration method described, coupled with low blanks and method detection limits (MDLs), demonstrates the effectiveness of the automated procedure using ethylenediaminetriacetic and iminodiacetate acid chelation exchange resin and multianalyte determination by ICP-MS for total and dissolved Ni, Cu, Zn, Cd, and Pb in marine water samples. Average CASS-5 recoveries using the online preconcentration method ($n = 9$) were $109\% \pm 7\%$, $104\% \pm 5\%$, $103\% \pm 7\%$, $101\% \pm 3\%$, and $86\% \pm 8\%$, respectively. The MDLs obtained from the automated method for Ni, Cu, Zn, Cd, and Pb were 3.3, 1.8, 13.5, 4, and 10 times lower, respectively, than for the Borohydride method. There were statistically significant differences between the methods for CASS-5 recoveries of Ni, Cu, Zn ($p < 0.0001$), and Pb ($p = 0.0024$). Comparison of methods gave high concordance ($r_C \geq 0.90$) between methods for total and dissolved Ni, Cu, Zn, and Pb, and total Cd.

The accuracy of marine trace metals determination-methodology chosen by research and compliance laboratories is both imperative and evolving. In marine biogeochemistry, trace metals in both dissolved and particulate form can serve as critical micronutrients, but also as potential toxicants to primary producers and marine organisms. The capacity to serve as a bio-limiting or toxic element often manifests over a fairly narrow free ion concentration range (Hudson and Morel 1993; Sunda and Huntsman 1998). While trace metal inputs to the coastal zone are driven largely by physical weathering of continents, anthropogenic inputs markedly disrupt free ion concentrations. To combat this, the U.S. Environmental Protection Agency (EPA) sets regulatory benchmarks for metals designated as priority pollutants (U.S. Environmental Protection Agency 2017); all metals discussed here have been designated priority under the Clean Water Act 40 CFR Part 423, Appendix A in seawater. EPA established environmental quality standards for

metals, as well as those of the European Economic Commission, are based on dissolved concentrations ($0.45 \mu\text{m}$ filtered) (European Economic Council 2008), while the Australian and New Zealand Environment and Conservation Council has established tiered environmental quality guidelines based on total and dissolved metal concentrations (Australian and New Zealand Environment and Conservation Council 2000). The narrow ranges of beneficial concentration make critical the ability to quantify trace metals of potential concern at very low concentrations in both transition areas and mixing zones, moving out from terrestrial run-off (e.g., harbor activities, storm water run-off, and sewage treatment plant outfalls).

Inductively coupled plasma mass spectrometry (ICP-MS) is widely used for trace metal detection in aqueous samples. Precise quantification of trace metal levels in seawater by ICP-MS must overcome spectral interferences caused by Ar support gas impurities and salts in the seawater matrix (Na, Cl, Mg, and Ca) which can lead to isobaric polyatomic ion introduction (Hirata et al. 2001). Naturally occurring high dissolved solids ($\sim 3\%$) in

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the seawater matrix are also problematic due to salt deposition on skimmer cones, resulting in flow restrictions that will impede both sensitivity and stability, leading to drift over an analytical run. Additionally, ionization suppression must be addressed due to high Na levels which pronounce the space-charge effect (Chapple and Byrne 1996; Rosland and Lund 1998). Some reduction of these interferences can be achieved by manipulation of instrument parameters, while others can be minimized by matrix-matched blank corrections or removal of the matrix via reductive precipitation. Dilution also attenuates these effects, but these methods all significantly decrease precision and sensitivity at ultra-trace levels. Consequently, analysts often seek the optimal method to separate the high salt background from the analytes of interest prior to analysis by ICP-MS.

A common analytical approach employed by the community for detection of Ni, Cu, Zn, Cd, and Pb in seawater samples is matrix separation coupled with analyte coprecipitation via borohydride reductive coprecipitation using a Fe and Pd mixture (Skogerboe et al. 1985; Nakashima et al. 1988; U.S. Environmental Protection Agency 1997a). This technique is an involved and lengthy process, which includes 15 h reaction time for precipitation as well as numerous manual steps, increasing the possibility of sample contamination. Additionally, the method requires reagent blank corrections to account for impurities introduced by precipitation reagents.

To decrease the contamination window and increase sample throughput, chelation exchange resins containing ethylenediaminetriacetic (EDTA) and iminodiacetic acids (IDA) have been developed to separate transition metals from matrices containing Na^+ , Cl^- , Mg^{2+} , and Ca^{2+} (Hirata et al. 1989; Warnken et al. 1999, 2000; Beck et al. 2002; Milne et al. 2010). These chelating ion exchange resins have been incorporated into a commercially available online preconcentration introduction system, seaFAST™ (Elemental Scientific, Omaha, Nebraska). The utility of this system for analysis of several elements by ICP-MS of open ocean seawater has recently been reported (Lagerström et al. 2013). The current study evaluates the utility of using this method in coastal waters where the concentrations range quite significantly from $\text{sub } \mu\text{g L}^{-1}$ to $10 \mu\text{g L}^{-1}$ levels.

This study compares borohydride reductive precipitation and seaFAST™ online preconcentration recoveries of certified seawater reference material (CASS-5) and filtered and unfiltered seawater samples from Sinclair (47.5486N, 122.6386W) and Dyes Inlets (47.6183N, 122.6896W) in Puget Sound, WA. The field samples were collected from naval dry-dock effluents, nearshore receiving waters, and marine reference areas as part of an ambient monitoring program conducted by the US Navy under an environmental quality improvement project (ENVVEST, Strivens et al. 2018). Field samples obtained from two collection campaigns conducted 07–08 April 2015 (spring) and 15–16 September 2015 (autumn) were analyzed using both methods. The major objective of the

current study was to validate the seaFAST™ preconcentration method for use on nearshore marine water samples, and to characterize any effect on long-term data sets.

The method-bridging implications provided in the current study define and quantify risk level to inform the approach for method transfer. In long-term data sets, shifts in baseline due to method improvement have probability to alter stability predictions in modeling. The implications of this, when assessing narrow acceptable ranges of trace metals in the marine environment, must be understood in studies of ecosystem health and accounted for in regulatory efforts, such as application of discharge mixing zones. The objective of method validation (demonstration of suitability) gauges quality, while the measure equivalency provided by the current study allows quantification of baseline shift and therefore a means of data-bridging.

Materials and procedures

Instrumentation

A Thermo Scientific™ iCAP™ Q ICP-MS (Waltham, Massachusetts, U.S.A.) was used for all measurements. The sample introduction system consisted of a Peltier-cooled spray chamber (Elemental Scientific, Omaha, Nebraska, U.S.A.), a low-flow PFA Nebulizer, semi-demountable concentric quartz torch with a 2.0 mm interior diameter (ID) quartz injector (Elemental Scientific, Omaha, Nebraska, U.S.A.), and Pt sample and skimmer cones (Thermo Scientific, Waltham, Massachusetts, U.S.A.) equipped with replaceable tip inserts to eliminate memory effect. A high-precision syringe driven system (seaFAST™) was used for sample introduction. The ICP-MS was operated in standard mode (STD) for borohydride reductive samples as Cl^- had already been eliminated from the matrix. A single collision cell mode with kinetic energy discrimination (KED), using pure He as collision gas, was used for seaFAST™ preconcentration samples to dually allow for direct injection for analysis of nonchelating trace metals.

The seaFAST™ system, referenced hereafter as the “Online” method, uses EDTA and IDA immobilized on a hydrophilic methacrylate polymer (part number CFN-0200) to preconcentrate transition elements. Under pH 6 conditions, maintained by an ammonium acetate solution, Na^+ , Cl^- , Mg^{2+} , and Ca^{2+} matrix ions pass through the columns, while the analytes of interest are chelated. After the preconcentration step, analytes are eluted using 1.5 M HNO_3 directly to the nebulizer. A (CF-M-0600) cleanup column was used to eliminate any trace analytes from both deionized water (DI) and buffer solutions.

Reagents

Concentrated Optima™ grade reagents (nitric, hydrochloric and acetic acids, and ammonium hydroxide) were purchased from Fisher (Pittsburgh, Pennsylvania, U.S.A.). High-purity DI water ($> 18 \text{ M}\Omega \text{ cm}$) was produced by a system composed of reverse osmosis and deionizing resins. Primary standards used for working standard mixtures, and an internal standard

premixed solution, were purchased from High-Purity Standards (Charleston, South Carolina, U.S.A.). Sodium borohydride and ammonium pyrrolidinedithiocarbamate (APDC) were purchased from Sigma-Aldrich (St. Louis, Missouri, U.S.A.).

Certified reference materials

In each analytical batch, the certified seawater reference material (CRM) CASS-5 was used to verify accuracy in a similar matrix. CASS-5 was obtained from the National Research Council of Canada (NRC, Ottawa, Ontario, Canada) and is marketed for use during analysis of nearshore seawater for trace metals. This water was collected from Halifax Harbor at a depth of 12 m, with a salinity of 33.5 psu. The field samples in the current study had a salinity range of 22.6–29.2 psu. The CASS-5 CRM was certified for all trace metals of interest in this study (Table 1).

Vessel cleaning

For sampling, Teflon™ bottles were cleaned with 50% (v/v) HNO₃ (Baker Instra-Analyzed®) at 85°C for 48 h. The Teflon™ was then triple rinsed with high-purity water and filled with 0.1% Optima™ grade HCl for 7 d to remove all residual traces of HNO₃. Prior to use, bottles were thoroughly rinsed with high-purity water and dried in a class-100 laminar flow clean-air hood.

For borohydride reductive coprecipitation, 50 mL polypropylene graduated centrifuge tubes with screw caps were cleaned using 80°C 10% (v/v) HNO₃ (Baker® Instra-Analyzed®) for 12 h followed by 80°C 1%/1% HCl/HNO₃ for an additional 12 h. This method was also applied to analytical tubes for the Online approach.

Sampling

Sampling followed ultra-clean collection procedures recommended for trace metals at EPA water quality criteria levels in EPA Method 1669 (U.S. Environmental Protection Agency 1996a). Surface grab (≤ 1 m depth) samples were collected

directly into the sample bottle with a nonmetallic sampling pole from a small boat. Subsurface collections at depths of 3–5 m were obtained with a Teflon-coated Go-Flo water sampler (General Oceanics, Miami, Florida, U.S.A.) suspended by nylon rope. Samples for dissolved metals were held at $< 4^\circ\text{C}$ and filtered through a 0.45 μm polyvinylidene fluoride membrane within 8 h of collection in a class 100 clean room. All samples were preserved to 0.2% Optima™ grade HNO₃ and stored for a minimum of 48 h prior to aliquoting.

Borohydride coprecipitation method

A modified version of EPA method 1640 (U.S. Environmental Protection Agency 1997a; Strivens et al. 2018) was performed within laminar air-flow benches, providing a class 100 working environment. Forty milliliters of sample were added to acid-cleaned centrifuge tubes. Addition of 0.3–0.5 mL of a Fe-Pd mixture (1:1 volume from 1000 $\mu\text{g mL}^{-1}$ stock) was followed by pH adjustment using ammonium hydroxide to 8.5, then, 0.5 mL of 5% (w/v) sodium borohydride solution was added. Prior to reductive precipitation, 0.25 mL of a 2% (w/v) APDC solution was added to the samples. Samples were allowed to settle overnight, then centrifuged at 3500 rpm for 30 min and decanted. Next samples were centrifuged for an additional 15 min and all remaining supernatant pipetted off. The addition of 0.1 mL of concentrated Optima™ grade HNO₃ to each Fe-Pd pellet was performed prior to placement in an oven at $80 \pm 2^\circ\text{C}$ for 20 min. Samples were then diluted to 5 mL volume with DI water for analysis. This procedure produced a sample preconcentration of eightfold. The calibration curve for this method included the Fe and Pd in the matrix to correct associated trace metal impurities. A set of method blanks was prepared and analyzed with each batch of samples to provide a characterization of the impurities in APDC, ammonium hydroxide, and sodium borohydride. Method blanks also provided a measure of any additional Al and Ni leaching from the polypropylene during the high temperature Fe-Pd reactions. The average blank value was subtracted from each sample value before reporting the data. Blank subtraction was also applied to laboratory control standards (LCS), CRMs, replicates, and spikes. In data analyzed prior to July 2015, this method was performed without addition of Fe-Pd to the curve and correction subtracted post analysis.

ESI seaFAST™ 2 online preconcentration method

The seaFAST™ 2, sample preconcentration system which fed into the ICP-MS, consisted of a 2 mL sample loop, a pre-packed EDTA/IDA preconcentration column on an SC-DX Fast and a Fast DX 3 valve system (Elemental Scientific, Omaha, Nebraska). Ten milliliters of undiluted seawater samples were loaded onto the SC-DX Fast auto-sampler. Prior to this step, metals samples were acidified to 1% HNO₃ and digested for 2 h at 85°C. The polytetrafluoroethylene loop of the seaFast™ 2 system was filled with 2 mL of sample and buffered via 2 M ammonium acetate, which then passed through the EDTA/IDA

Table 1. Certified values of CASS-5 seawater CRM.

Element	Certified value ($\mu\text{g L}^{-1}$)	Method of determination
Ni	0.33 ± 0.023	*,†,‡
Cu	0.38 ± 0.028	*,†,‡
Zn	0.719 ± 0.068	*,†,§,
Cd	0.0215 ± 0.0018	*,†,‡,
Pb	0.011 ± 0.002	†,

*Immobilized ligand separation, determination by graphite furnace atomic adsorption.

†Immobilized ligand separation, determination by isotope dilution ICP-MS.

‡Reductive precipitation separation, determination by graphite furnace atomic absorption spectroscopy.

§Immobilized ligand separation, determination by ICP-MS.

||Immobilized ligand separation, determination by inductively coupled plasma time-of-flight mass spectrometer.

Table 2. Fast operating parameters.

Step	Fast valves (action: method timer)	Syringe action (flow rate: volume)	Summary
(1a) Precheck	V1: Load V2: Load V3: Load		Fast valves are activated
(1b) Start preconcentration		S1: 2500 $\mu\text{L min}^{-1}$: 8000 μL S2: 730 $\mu\text{L min}^{-1}$: 2333 μL S4: 200 $\mu\text{L min}^{-1}$: continuous	(S1) DI water and (S2) buffer rinse preconcentration column; (S4) eluent is directed to the nebulizer
(1c) Load 2 mL sample loop for 2 s	V2: Inject: 10 s		After loop loading, the sample is directed through preconcentration column at same time as (S2) buffer
(2) DI wash	V2: Load: 170 s	S3: 200 $\mu\text{L min}^{-1}$: continuous	Salt matrix is rinsed from preconcentration column using (S1) DI water
(3) Elution	V1: Inject: 190 s	S3: 200 $\mu\text{L min}^{-1}$: continuous S4: 750 $\mu\text{L min}^{-1}$: continuous	Preconcentrated metals are back-eluted to the nebulizer by the (S3/S4) diluent/carrier eluent
(4a) Loop rinse	V2: Load		Probe moves to rinse Sta. 1 and 2 for 3 s each.
(4b) Column cleanup	V1: Inject: 320 s	S3: 3000 $\mu\text{L min}^{-1}$: continuous S4: 1500 $\mu\text{L min}^{-1}$: continuous	(S3) eluent is back-eluted through the preconcentration column; (S4) eluent is back eluted through the trace metals cleanup column
(4c) Condition columns	V1: Load	S1: 2500 $\mu\text{L min}^{-1}$: 2000 μL S2: 833 $\mu\text{L min}^{-1}$: 667 μL	DI water (S1) and buffer (S2) pass through the preconcentration column to condition for next sample
(4d) Fill syringes		S1: 20,000 $\mu\text{L min}^{-1}$: continuous S2: 10,000 $\mu\text{L min}^{-1}$: continuous S3: 10,000 $\mu\text{L min}^{-1}$: continuous S4: 10,000 $\mu\text{L min}^{-1}$: continuous	Syringes are refilled
(4e) Predispense		S1: 3000 $\mu\text{L min}^{-1}$: 600 μL S2: 1000 $\mu\text{L min}^{-1}$: 150 μL S3: 1000 $\mu\text{L min}^{-1}$: 150 μL S4: 1000 $\mu\text{L min}^{-1}$: 150 μL	Lines are primed
	Total method timer time: 480 s		

column. The pH was held constant by continuous rinsing with 2 M ammonium acetate solution. The interstitial volume of the line was rinsed with DI water to remove the residual components of the sample matrix. Meanwhile, the sample on the column was flushed to remove Na^+ , Cl^- , Mg^{2+} , and Ca^{2+} matrix ions. The elution was achieved with 1.5 M HNO_3 which back-flushed the analytes of interest to the PFA-ST nebulizer. The base method for this process is provided in U.S. Environmental Protection Agency (1997b); operating parameters for the current study are given in Table 2. The calibration curve for this method, using High-Purity Standards diluted with 1% HNO_3 , was generated using the preconcentration method, thereby incorporating any signal from the reagents.

ICP-MS method

The ICP-MS procedure (Strivens et al. 2018) was based on EPA Method 1638 (U.S. Environmental Protection Agency 1996b). Internal standards (^{45}Sc , ^{73}Ge , ^{89}Y , ^{115}In , and ^{185}Re)

were used to account for variations in sensitivity over the sample run for analysis of borohydride coprecipitated samples; for the Online method, peak acquisition drift was monitored by quality control (QC) samples as internal standards do not preconcentrate. QC samples for both methods included: a 1% HNO_3 acidified DI blank, a LCS, two matrix spikes (1 $\mu\text{g L}^{-1}$ or 2 $\mu\text{g L}^{-1}$ and 5 $\mu\text{g L}^{-1}$), two matrix spike duplicates, a sample duplicate (DUP), and a CASS-5 CRM. The LCS was a sample of 0.45 μm filtered Sequim Bay (48.077759 N, 123.045005 W) seawater spiked with 2 $\mu\text{g L}^{-1}$ of each analyte, carried through the entire preparation scheme of each preconcentration method, with the purpose of determining whether the method was within accepted control limits. One suite of the aforementioned QC samples was run for every 20 environmental samples. Operating conditions of the iCAP Q are detailed in Table 3. Isotopes monitored were ^{27}Al , ^{60}Ni , ^{62}Ni , ^{63}Cu , ^{65}Cu , ^{64}Zn , ^{66}Zn , ^{68}Zn , ^{112}Cd , ^{114}Cd , ^{206}Pb , and ^{208}Pb . The calibration curves for initial analyses by the Borohydride method

extended to $50 \mu\text{g L}^{-1}$; the extent of the consequent Online analyses were adjusted to cover the determined ranges with a minimum extent up to $5 \mu\text{g L}^{-1}$.

Table 3. ICP-MS instrument operating conditions.

Parameter	Value
RF power	1550 W
Coolant airflow	14 L min ⁻¹
Auxiliary airflow	0.8 L min ⁻¹
Carrier airflow	1.05 L min ⁻¹
Nebulizer	0.3 mL min ⁻¹
Spray chamber	Buffered cyclonic
Detector mode	Pulse and analog
Dwell times	0.01–0.04 s
Sweeps	25
Sample depth	5 mm

Table 4. Method detection limits determined for this study using the iCapQ ICP-MS and Sequim Bay seawater. Units are expressed as $\mu\text{g L}^{-1}$.

Method	Ni	Cu	Zn	Cd	Pb
Borohydride STD mode	0.050	0.014	0.175	0.004	0.0040
Online preconcentration, KED mode	0.015	0.008	0.013	0.001	0.0004

Table 5. Quality control comparison from sampling events spring and autumn, using both borohydride reductive coprecipitation and Online preconcentration methods.

	QC parameter	Method	Spike ($\mu\text{g L}^{-1}$)	<i>n</i>	Average recoveries*				
					Ni	Cu	Zn	Cd	Pb
Spring campaign	Low spike	Borohydride	1	10	82.7 ± 6.3	83.5 ± 6.3	86.2 ± 11.0	94.1 ± 3.0	89.5 ± 1.8
		Online	2	4	101.9 ± 12.3	102.5 ± 9.1	105.6 ± 8.6	100.4 ± 4.6	100.1 ± 2.4
	High spike	Borohydride	5	6	79.9 ± 3.2	80.4 ± 3.9	71.8 ± 4.4	91.9 ± 4.0	88.6 ± 2.7
		Online	5	—	—	—	—	—	—
	SB LCS [†]	Borohydride	2	5	81.7 ± 4.7	82.5 ± 4.3	83.3 ± 6.7	92.3 ± 1.9	87.6 ± 1.4
		Online	2	4	106.3 ± 7.2	106.4 ± 6.2	105.5 ± 7.3	102.9 ± 2.7	101.8 ± 3.0
	Replicate RPD	Borohydride	—	5	1.7 ± 1.8	1.8 ± 1.6	5.1 ± 2.7	3.0 ± 1.6	2.6 ± 1.5
		Online	—	5	1.4 ± 0.5	1.6 ± 0.7	1.5 ± 1.1	1.0 ± 0.9	1.4 ± 1.9
Autumn campaign	Low spike	Borohydride	1	10	80.9 ± 9.2	81.3 ± 8.4	74.7 ± 24.9	93.2 ± 2.1	92.2 ± 2.2
		Online	2	4	106.3 ± 1.9	105.5 ± 0.5	105.0 ± 0.7	102.3 ± 0.8	98.4 ± 1.8
	High spike	Borohydride	5	10	83.1 ± 8.6	82.4 ± 8.5	64.2 ± 27.8	91.8 ± 4.3	91.2 ± 4.2
		Online	5	4	101.9 ± 4.5	99.7 ± 2.0	102.5 ± 2.4	102.7 ± 1.6	100.0 ± 1.4
	SB LCS [†]	Borohydride	2	5	87.9 ± 3.2	85.9 ± 4.5	87.5 ± 4.1	94.2 ± 0.9	93.3 ± 2.0
		Online	2	5	110.5 ± 6.0	107.4 ± 4.1	108.5 ± 2.3	104.4 ± 2.0	100.5 ± 2.5
	Replicate RPD	Borohydride	—	5	2.5 ± 2.2	2.1 ± 2.1	2.7 ± 1.7	1.8 ± 1.7	3.3 ± 4.1
		Online	—	3	0.8 ± 1.0	1.2 ± 0.8	1.0 ± 0.4	0.8 ± 0.4	1.8 ± 0.2

*All values are reported as percent recovery with the exception of the replicates as RPD.

[†]SB LCS is a Sequim Bay laboratory control sample spiked at $2 \mu\text{g L}^{-1}$ with an SB Blank correction applied.

Statistical data evaluation

Data were imported into R-Studio (v98.1091, r-studio.com, Boston, Massachusetts) running R (v3.01.1, R Foundation for Statistical Computing, www.r-project.org) for statistical analysis. A two-way ANOVA was conducted to evaluate the null hypothesis that there were no differences between methods and events for the analysis of CRMs for the metals of interest:

$$F = \text{aov}(Y \sim \text{Method}^* \text{Event}, \text{data} = \text{CRMdata}) \quad (1)$$

where Y = variable of interest; Method = Borohydride or Online; Event = Spring or Autumn campaign; and CRMdata = data set of CRM results with $n = 5$ or 4 CRM measurements for each method and event. The null hypothesis was rejected if $p \leq 0.05$. Box and whisker plots for each metal were constructed to visualize statistical comparisons, compare to certified values, and evaluate the magnitude of the differences between the methods.

Results from the field sampling campaigns were compared by plotting the difference between methods ($D = Y_B - Y_O$) vs. the mean ($\mu = [Y_B + Y_O]/2$) of the methods (Altman and Bland 1983; Bland and Altman 1986) to evaluate any constant or proportional bias. The agreement between methods was then determined by calculating the correlation coefficient (r), concordance coefficient (r_C) (Magari 2002; Watson and Petrie 2010), and Gold-standard correlation (r_G) (St. Laurent 1998; Magari 2002) as:

$$r = \text{CORREL}(Y_{Bi} : Y_{Bn}, Y_{Oi} : Y_{On}) \quad (2)$$

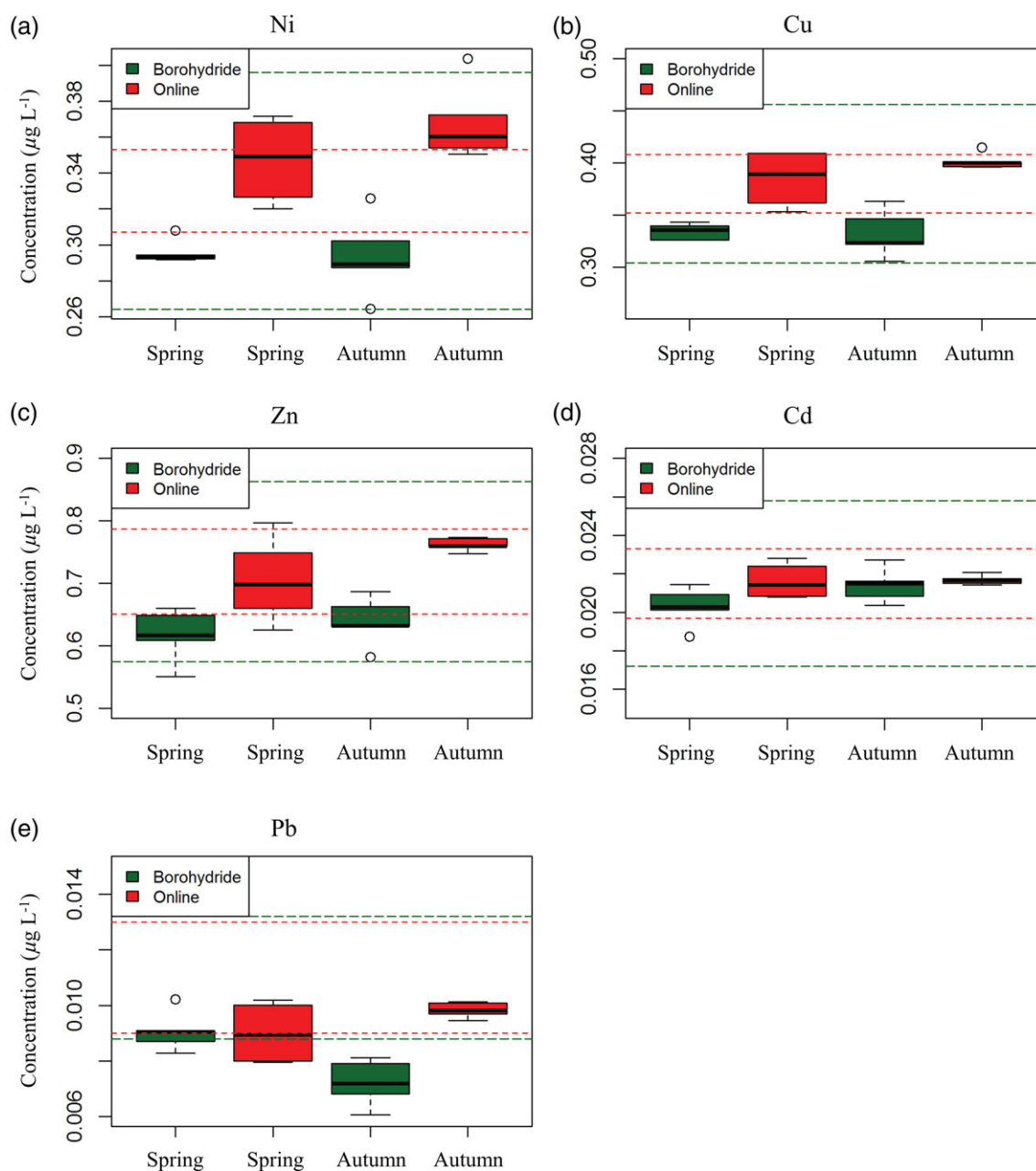


Fig. 1. (a–e) Box and whisker plots of CASS-5 recoveries ($\mu\text{g L}^{-1}$) for Ni, Cu, Zn, Cd, and Pb during analysis of sampling events, spring and autumn, using the Borohydride and Online methods. Certified concentrations are signified by red dashed lines, and 20% difference QC limits by green dashed lines.

Table 6. Results of two-way ANOVA for method and event for each metal analyzed in CASS-5 CRM samples.

<i>p</i>	Ni	Cu	Zn	Cd	Pb
Method	<0.0001	<0.0001	<0.0001	0.0541	0.0024
Event	0.3620	0.4430	0.0738	0.1283	0.1260
Method*Event	0.2320	0.2900	0.3876	0.1955	0.0022

$$r_C = \frac{2S_{OB}}{S_B^2 + S_O^2 + (O-B)^2} \quad (3)$$

$$r_G = \frac{1}{1 + \frac{S_D}{S_B^2(n-1)}} \quad (4)$$

where Y_{Bi} and Y_{Oi} were the measurement results using the Borohydride and Online methods, respectively; n was the

Table 7. Trace metal impurities in Fe and Pd reagent solutions.

Study	Analyte	Lot#	Trace metal impurity ($\mu\text{g L}^{-1}$)*				
			Ni	Cu	Zn	Cd	Pb
Spring	Fe	1,109,003	10	0.01	0.02	0.02	0.02
	Pd	1,112,902	1	3	1	0.01	0.05
	Total		11	3.01	1.02	0.03	0.07
Autumn	Fe	1,503,405	2	1	2	0.02	2
	Pd	1,504,103	7	7	2	0.1	0.03
	Total		9	8	4	0.12	2.03
	Difference		-2	4.99	2.98	0.09	1.96

*Values taken from High Purity Standards Certificate of Analysis for 99.99% Fe and Pd.

Table 8. Percent recovery of CASS-5 certified analytes using the Borohydride and Online methods for the current study. Results from the long-term data set are also presented.

Method	<i>n</i>	Ni	Cu	Zn	Cd	Pb
Study recovery						
Borohydride	10	89.3 \pm 4.8	87.7 \pm 4.2	87.4 \pm 5.6	97.0 \pm 5.0	74.1 \pm 11.1
Online	9	108.8 \pm 7.3	103.8 \pm 5.3	102.5 \pm 7.4	100.7 \pm 2.9	86.1 \pm 7.9
Long-term data set (2009–2015)						
Borohydride	*	100.3 \pm 9.3	108.2 \pm 12.9	101.6 \pm 38.1	114.4 \pm 15.2	97.5 \pm 35.2
Borohydride†	*	96.2 \pm 7.9	97.3 \pm 17.2	92.5 \pm 38.2	113.9 \pm 14.9	89.3 \pm 33.3
Long-term data set (2015–2018)						
Online	39	108.4 \pm 5.4	101.5 \pm 4.6	103.0 \pm 4.7	104.8 \pm 5.0	95.4 \pm 12.8

*Ni = 80; Cu, Zn, Cd, Pb = 102.

†Reagent blank corrected.

number of paired measurements; S_{OB} was the covariance between the Online and Borohydride method; S_B^2 was the variance of the Borohydride method; S_O^2 was the variance of the Online method; O and B were the means of the Online and Borohydride methods, respectively; and $S_D = \Sigma D^2$. These indices of reliability (Watson and Petrie 2010) were calculated for each metal grouped by dissolved and total results.

Assessment

Blanks and accuracy

For DI water blanks bracketing sample groups, recoveries for online preconcentration were below the method detection limits (MDLs) for all analytes given; matrix corrected borohydride reductive coprecipitation method blanks were less than two times the MDLs given in Table 4. MDLs were determined using seven replicates, of 0.45 μm filtered Sequim Bay seawater spiked to attain a concentration 2–10 times the estimated detection limit, and calculated in accordance with 40 CFR Part 136, Appendix B. The MDLs obtained from the Online method for Ni, Cu, Zn, Cd, and Pb were 3.3, 1.8, 13.5, 4, and 10 times lower, respectively, than the Borohydride method. LCS values, as with other QC samples, recovered with consistently greater accuracy using Online preconcentration. This trend, as well as

an overall higher yield, is seen throughout the comparison of recoveries, and is also highlighted in the spike recoveries in Table 5. One line of reasoning is that the Borohydride method produces lower recoveries due to trace metal analytes coprecipitation sensitivities to pH, while Online preconcentration allows for a much wider range (Biller and Bruland 2012). Another factor to consider for Online accuracy is loading rate and volume of the preconcentration column; Rapp et al. (2017), for example, obtained significantly low recoveries for Ni using a resin volume of 15 μL in contrast to the 200 μL column in the current study. Sample replicates for each study recovered all analytes within 9% as relative percent difference (RPD).

Precision

The results of CRM analysis are shown in Fig. 1, the results of the ANOVA are presented in Table 6 for both methods and sampling events. There were statistically significant differences ($p < 0.0001$) between the methods for Ni, Cu, and Zn, statistical differences between methods for Pb ($p = 0.0024$), and minor differences for Cd ($p = 0.054$). For Ni, the Online method had higher accuracy while the Borohydride method was more precise, and both methods fell within the QC limits with the exception of one outlier from the autumn Online analysis (Fig. 1a). The Online method was remarkably accurate

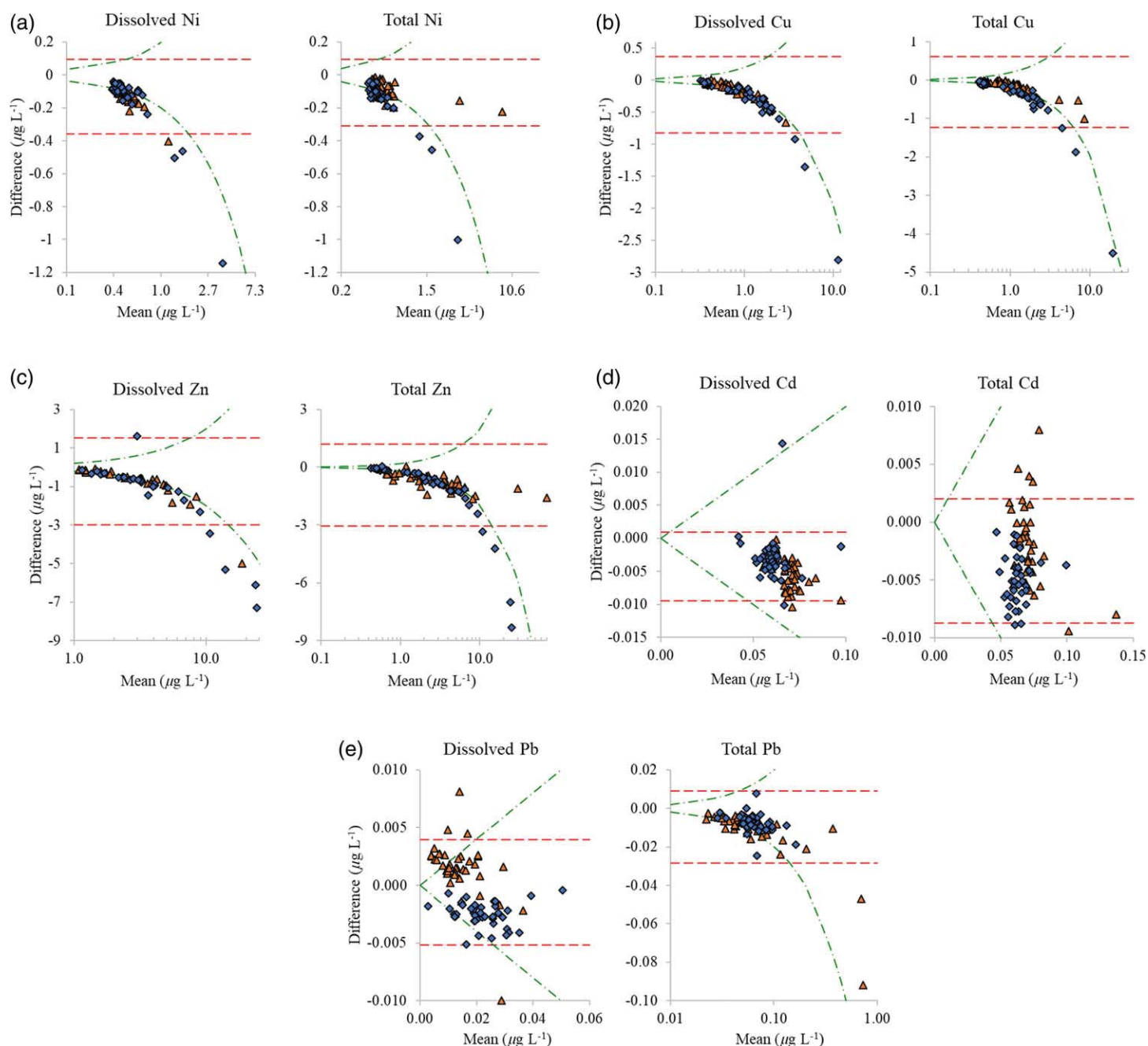


Fig. 2. (a–e) Difference plots of dissolved and total trace metal concentrations determined using borohydride reductive preconcentration vs. the chelating ion exchange method (n provided in Table 9). Triangles represent spring samples, diamonds represent autumn, red dashed lines indicate the 95th percentile, and green dash-dot lines signify 20% variance from unity.

for Cu and Zn, and both methods were exceptionally accurate for Cd (Fig. 1b–d). There were no statistical differences ($p > 0.05$) for any of the metals between events, but there was a statistically significant interaction between method and event for Pb ($p = 0.0022$; Table 6; Fig. 1e).

The box and whisker plots show that the Borohydride method had lower recoveries and was less accurate for Ni, Cu, and Zn than the Online method (Fig. 1). The CRM recoveries

observed for the Borohydride method for the two events displays the significance of purity levels of reagents available on the market. Different batches of Fe-Pd solution for the Borohydride method were used on the two events. The two batches had significantly different impurities for the metals of interest (Table 7). Note that the Pb concentration in the Fe solution used for autumn sampling was 100-fold higher than the Fe solution used for spring event. This radical concentration

Table 9. The number of samples, mean percent differences (PD \pm standard deviation), correlation coefficient (r), concordance (r_C), and Gold-Standard (r_G) statistics calculated between methods for metals grouped by dissolved and total results.

				<u>Correlation</u>	<u>Concordance</u>	<u>Gold-standard</u>
		<i>n</i>	Mean PD	<i>r</i>	<i>r</i> _C	<i>r</i> _G
Ni	Dissolved	80	23% ± 6%	0.997	0.897	0.756
	Total	81	18% ± 9%	0.994	0.987	0.972
Cu	Dissolved	81	17% ± 7%	0.999	0.956	0.893
	Total	81	17% ± 9%	0.997	0.966	0.920
Zn	Dissolved	81	13% ± 18%	0.997	0.942	0.858
	Total	81	27% ± 14%	0.993	0.985	0.968
Cd	Dissolved	81	6% ± 5%	0.947	0.847	0.720
	Total	81	5% ± 5%	0.962	0.924	0.861
Pb	Dissolved	81	−2% ± 23%	0.965	0.951	0.896
	Total	81	13% ± 7%	0.999	0.991	0.981

difference offers an explanation for the drop in Pb recovery for the Borohydride method between sampling events (Fig. 1e). The CASS-5 Pb recovery with the Borohydride method was problematic due to reagent impurities holding the MDL near the CRM value, and matrix correcting at levels nearly twice the certified value; 0.0210 $\mu\text{g L}^{-1}$ during the Autumn event.

The historical accuracy of the borohydride reduction method for the project associated to these samples is given in Table 8, showing determinations of the CASS-5 CRM during, preanalytical, and postanalytical method shift, and highlighting the disadvantage associated with the weight of correction levels. Historically, good average accuracy has been obtained with the blank corrected average Borohydride recoveries, ranging from a low of 89.3% for Pb to a high of 113.9% for Cd. All Borohydride determined analytes (2009–2015) had a 95% confidence level within 20% variation for accuracy to the CRM both preblank and postblank correction. Precision and repeatability are given by the percent standard deviation showing that the magnitude of dispersion is generally unaffected by blank correcting, leaving the average recovery ranges outside of a 20% limit to the certified reference values for Zn, Cd, and Pb.

Environmental sample analysis

The comparison of the results obtained by the Online and Borohydride methods from the analysis of field samples collected from Sinclair and Dyes Inlets is shown in Fig. 2 as the difference between the methods as a function of the mean of paired measurements using the Borohydride and Online methods for total and dissolved fraction measurements of samples collected from two sampling events. The difference plots provide a better comparison of the methods than correlation and regression analysis because methods designed to measure the same thing will be invariably highly correlated (Altman and Bland 1983; Bland and Altman 1986) and regression analysis will not necessarily reveal any constant or proportional bias between the methods (Magari 2002; Watson

and Petrie 2010). A summary of the indices of reliability calculated for each metal grouped by dissolved, and total results are provided in Table 9.

For dissolved and total Ni, Cu, and Zn, there were constant proportional biases toward the Online method with the average PD of dissolved Ni and total Zn exceeding 20% (Table 9). The dissolved and total Cd results remained well within 20% of unity with a constant bias of $\sim 0.003 \mu\text{g L}^{-1}$ toward the Online method. Dissolved Pb was near unity, with spring results biased toward the Borohydride method and the autumn samples biased toward the Online method. Total Pb showed a proportional bias toward the Online method.

As expected, there were substantial correlations ($r \geq 0.95$) between the methods for all metals. There was almost perfect concordance ($r_C \geq 0.99$) between methods for Total Pb, and substantial concordance ($r_C \geq 0.95$) for total Ni, Cu, Zn, and dissolved Ni, Cu, and Pb. Dissolved Cd had the minimum concordance; indicating 85% agreement (Table 9). The r_C values indicate variation from best fit and shift from unity. Dissolved Cd variation in r_C was 9% linearity and 6% unity fitment; the outlier observed in Fig. 2d had minimal impact on this ratio. In relation to r , dissolved Ni and Cd displayed the most significant shift (10%). When assessing total Ni, it becomes apparent that the two maximal spring samples in Fig. 2a result in the shift in r_C between total and dissolved fractions; sans these points both concordance values would display 4% variance from linearity (r) and 6% from unity fitment; this is true also of the delta r_C in Zn species and is likely a result of inhomogeneity of particulates in total sample aliquots.

The Gold-standard statistic, using Borohydride as the gold standard, was substantial ($r_G \geq 0.95$) for total Ni, Zn, and Pb and moderate ($r_G \geq 0.90$) for total Cu; for other measures, the variation between methods was significant in relation to the range of ambient levels. The r_G is provided as a measure of the strength of r_C over the ambient ranges of analytes tested.

In general, the shifts in method recoveries of field samples reflect the trend of increased recovery by the Online method equivalent to that of the QC samples. This is likely due to a combination of overcorrecting the Borohydride method for impurities seen in Table 8, and loss of nanoparticles in the Borohydride method supernatant. Based on CRM percent differences, these shifts are correcting, not skewing the data, with Ni being a questionable exception. The shifts given as mean PD \pm standard deviation in Table 9 allow decisions of confidence in method replacement based on individual project's historical ranges and thresholds. While these biases are minimal they must be accounted for when combining data-sets using the differing methods.

Discussion

The borohydride reductive coprecipitation method from EPA Method 1640 is commonly used to quantify Ni, Cu, Zn, Cd, and Pb in seawaters via analysis by ICP-MS. However, the chelating reagents required to induce precipitation contain trace impurities that require a reagent blank correction to accurately represent the trace metal concentrations in ambient seawaters. The current study demonstrates that moving to an automated online procedure using EDTA/IDA resulted in good reproducibility compared to the borohydride reductive precipitation method, and eliminated the systematic negative bias generated during the Fe-Pd reagent blank correction. The comparability of the methods was demonstrated over a range of trace metal concentrations representative of a nearshore industrial harbor for both total recoverable metals and the dissolved fraction. The greatest benefit of a transition to EDTA/IDA is that detection limits for analytes are no longer tied to the level of impurities in the chelating reagents; thus, matrix interferences are eliminated without additional data corrections being necessary. This allows for more quantitative measurements, at lower detection limits, that are more relevant to ambient trace metal concentrations in seawaters. The systematic data bias ranged from -2% to $+27\%$ when comparing the Borohydride to the Online method. The robust results of the side by side method comparison demonstrate that EDTA/IDA is the preferred method. As research programs utilize empirical modeling to determine trace metal fate and transport, this online method should be considered capable of providing highly precise data that are needed to conduct low-level trace metal modeling and toxicity assessment in near-shore and marine coastal systems.

References

- Altman, G., and J. M. Bland. 1983. Measurement in medicine: The analysis of method comparison studies. *Statistician* **32**: 307–317. doi:[10.2307/2987937](https://doi.org/10.2307/2987937)
- Australian and New Zealand Environment and Conservation Council. 2000. Australian and New Zealand guidelines for fresh and marine water quality. Australian Water Association.
- Beck, N. G., R. P. Franks, and K. W. Bruland. 2002. Analysis for Cd, Cu, Ni, Zn, and Mn in estuarine water by inductively coupled plasma mass spectrometry coupled with an automated flow injection system. *Anal. Chim. Acta* **455**: 11–22. doi:[10.1016/S0003-2670\(01\)01561-6](https://doi.org/10.1016/S0003-2670(01)01561-6)
- Biller, D. V., and K. W. Bruland. 2012. Analysis of Mn, Fe, Co, Ni, Cu, Zn, Cd, and Pb in seawater using the Nobias-chelate PA1 resin and magnetic sector inductively coupled plasma mass spectrometry (ICP-MS). *Mar. Chem.* **130**: 12–20. doi:[10.1016/j.marchem.2011.12.001](https://doi.org/10.1016/j.marchem.2011.12.001)
- Bland, J. M., and D. G. Altman. 1986. Statistical methods for assessing agreement between two pairs of clinical measurement. *Lancet* **327**: 307–310. doi:[10.1016/S0140-6736\(86\)90837-8](https://doi.org/10.1016/S0140-6736(86)90837-8)
- Chapple, G., and J. P. Byrne. 1996. Direct determination of trace metals in sea-water using electrothermal vaporization inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* **11**: 549–553. doi:[10.1039/JA9961100549](https://doi.org/10.1039/JA9961100549)
- European Economic Council. 2008. Directive 2008/105/EC of the European Parliament and of the council of 16 December 2008. Annex I: Environmental quality standards for priority substances and certain other pollutants. Official Journal of the European Union. 24.12.2008: L 348/84–348/97.
- Hirata, S., K. Honda, and T. Kumamaru. 1989. Trace metal enrichment by automated on-line column preconcentration for flow-injection atomic absorption spectrometry. *Anal. Chim. Acta* **221**: 65–76. doi:[10.1016/S0003-2670\(00\)81939-X](https://doi.org/10.1016/S0003-2670(00)81939-X)
- Hirata, S., Y. Ishida, M. Aihara, K. Honda, and O. Shikino. 2001. Determination of trace metals in seawater by on-line column preconcentration inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* **438**: 205–214. doi:[10.1016/S0003-2670\(01\)00859-5](https://doi.org/10.1016/S0003-2670(01)00859-5)
- Hudson, R. J., and F. M. Morel. 1993. Trace metal transport by marine microorganisms: Implications of metal coordination kinetics. *Deep-Sea Res. Part I Oceanogr. Res. Pap.* **40**: 129–150. doi:[10.1016/0967-0637\(93\)90057-A](https://doi.org/10.1016/0967-0637(93)90057-A)
- Lagerström, M. E., M. P. Field, M. Séguet, L. Fischer, S. Hann, and R. M. Sherrell. 2013. Automated on-line flow-injection ICP-MS determination of trace metals (Mn, Fe, Co, Ni, Cu and Zn) in open ocean seawater: Application to the GEO-TRACES program. *Mar. Chem.* **155**: 71–80. doi:[10.1016/j.marchem.2013.06.001](https://doi.org/10.1016/j.marchem.2013.06.001)
- Magari, R. T. 2002. Statistics for laboratory method comparison studies. *BioPharm* **2002**: 28–32. doi:[10.1016/j.marchem.2013.06.001](https://doi.org/10.1016/j.marchem.2013.06.001)
- Milne, A., W. Landing, M. Bizimis, and P. Morton. 2010. Determination of Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb in seawater using high resolution magnetic sector inductively coupled mass spectrometry (HR-ICP-MS). *Anal. Chim. Acta* **665**: 200–207. doi:[10.1016/j.aca.2010.03.027](https://doi.org/10.1016/j.aca.2010.03.027)

- Nakashima, S., R. E. Sturgeon, S. N. Willie, and S. S. Berman. 1988. Determination of trace elements in sea water by graphite-furnace atomic absorption spectrometry after pre-concentration by tetrahydroborate reductive precipitation. *Anal. Chim. Acta* **207**: 291–299. doi:[10.1016/S0003-2670\(00\)80804-1](https://doi.org/10.1016/S0003-2670(00)80804-1)
- Rapp, I., C. Schlosser, D. Rusiecka, M. Gledhill, and E. P. Achterberg. 2017. Automated preconcentration of Fe, Zn, Cu, Ni, Cd, Pb, Co, and Mn in seawater with analysis using high-resolution sector field inductively-coupled plasma mass spectrometry. *Anal. Chim. Acta* **976**: 1–13. doi:[10.1016/j.aca.2017.05.008](https://doi.org/10.1016/j.aca.2017.05.008)
- Rosland, E., and W. Lund. 1998. Direct determination of trace metals in sea-water by inductively coupled plasma mass spectrometry. *J Anal Atom Spectrom* **13**: 1239–1244. doi:[10.1039/A804350G](https://doi.org/10.1039/A804350G)
- Skogerboe, R. K., W. A. Hanagan, and H. E. Taylor. 1985. Concentration of trace elements in water samples by reductive precipitation. *Anal. Chem.* **57**: 2815–2818. doi:[10.1021/ac00291a016](https://doi.org/10.1021/ac00291a016)
- St. Laurent, R. T. 1998. Evaluating agreement with a gold standard in method comparison studies. *Biometrics* **54**: 537–545. doi:[10.2307/3109761](https://doi.org/10.2307/3109761)
- Strivens, J. E., R. K. Johnston, N. Schlafer, and J. M. Brandenberger. 2018. ENVVEST Ambient Monitoring Program: In-Progress Summary 2009–2017. PNNL-28116, prepared for the Puget Sound Naval Shipyard and Intermediate Maintenance Facility under Project ENVVEST by the Pacific Northwest National Laboratory, Marine Sciences Laboratory, Sequim, Washington. Final Report, Sept. 2018, 53pp + appendices.
- Sunda, W. G., and S. A. Huntsman. 1998. Processes regulating cellular metal accumulation and physiological effects: Phytoplankton as model systems. *Sci. Total Environ.* **219**: 165–181. doi:[10.1016/S0048-9697\(98\)00226-5](https://doi.org/10.1016/S0048-9697(98)00226-5)
- U.S. Environmental Protection Agency. 1996a. Method 1669: Sampling ambient water for determination of metals at EPA water quality criteria levels. U.S. Environmental Protection Agency, Office of Water.
- U.S. Environmental Protection Agency. 1996b. Method 1638: Determination of trace elements in ambient waters by inductively coupled plasma – mass spectrometry. US EPA Office of Water.
- U.S. Environmental Protection Agency. 1997a. Method 1640: Determination of trace elements in water by preconcentration and inductively coupled plasma-mass spectrometry. Office of Water and Office of Science and Technology.
- U.S. Environmental Protection Agency. 1997b. Method 200.10: Determination of trace elements in marine waters by on-line chelation preconcentration and inductively coupled plasma – mass spectroscopy, p. 15. Revision 1.6. Office of Research and Development.
- U.S. Environmental Protection Agency. 2017. National recommended water quality criteria - aquatic life criteria table. Office of Water. Available from <https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table>
- Warnken, K., G. Gill, L. Griffin, and P. Santschi. 1999. Trace metal analysis of natural waters by ICP-MS with on-line preconcentration and ultrasonic nebulization. *J Anal Atom Spectrom* **14**: 247–252. doi:[10.1039/A806822D](https://doi.org/10.1039/A806822D)
- Warnken, K. W., D. Tang, G. A. Gill, and P. H. Santschi. 2000. Performance optimization of a commercially available iminodiacetate resin for the determination of Mn, Ni, Cu, Cd and Pb by on-line preconcentration inductively coupled plasma-mass spectrometry. *Anal. Chim. Acta* **423**: 265–276. doi:[10.1016/S0003-2670\(00\)01137-5](https://doi.org/10.1016/S0003-2670(00)01137-5)
- Watson, P. F., and A. Petrie. 2010. Method agreement analysis: A review of correct methodology. *Theriogenology* **73**: 1167–1179. doi:[10.1016/j.theriogenology.2010.01.003](https://doi.org/10.1016/j.theriogenology.2010.01.003)

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Conflict of Interest

None declared.

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